jraas

Journal of the Renin-Angiotensin-Aldosterone System
January-March 2019: 1–6

DOI: 10.1177/1470320319827449
journals.sagepub.com/home/jra
SAGE

# The selective mineralocorticoid receptor modulator AZD9977 reveals differences in mineralocorticoid effects of aldosterone and fludrocortisone

**Krister Bamberg[1], Lena William-Olsson[1], Ulrika Johansson[1], Rasmus Jansson-Löfmark[2] and Judith Hartleib-Geschwindner[1]**

## Abstract

**Introduction:** AZD9977 is a novel mineralocorticoid receptor (MR) modulator, which in preclinical studies demonstrated organ protection without affecting aldosterone-regulated urinary electrolyte excretion. However, when tested in humans, using fludrocortisone as an MR agonist, AZD9977 exhibited similar effects on urinary $Na^+/K^+$ ratio as eplerenone. The aim of this study is to understand whether the contradictory results seen in rats and humans are due to the mineralocorticoid used.

**Materials and methods:** Rats were treated with single doses of AZD9977 or eplerenone in combination with either aldosterone or fludrocortisone. Urine was collected for five to six hours and total amounts excreted $Na^+$ and $K^+$ were assessed.

**Results:** AZD9977 dose-dependently increased urinary $Na^+/K^+$ ratio in rats when tested against fludrocortisone, but not when tested against aldosterone. Eplerenone dose-dependently increased urinary $Na^+/K^+$ ratio when tested against fludrocortisone as well as aldosterone.

**Conclusions:** The data suggest that the contrasting effects of AZD9977 on urinary electrolyte excretion observed in rats and humans are due to the use of the synthetic mineralocorticoid fludrocortisone. Future clinical studies are required to confirm the reduced electrolyte effects of AZD9977 and the subsequent lower predicted hyperkalemia risk.

## Keywords

Aldosterone, AZD9977, fludrocortisone, mineralocorticoid receptor ligand, urinary electrolytes

Date received: 3 July 2018; accepted: 17 December 2018

## Introduction

Pathophysiological activation of the mineralocorticoid receptor (MR) promotes inflammation, oxidative stress and fibrosis and ultimately leads to target organ dysfunction and an increased risk of cardiovascular and renal disease.[1,2] MR antagonists (MRAs) provide effective treatment for heart failure (HF),[3,4] and several MRAs are currently in development for treatment of chronic kidney disease.[5–7] A limitation with current MRAs is the mechanism-based risk of hyperkalemia, since MRAs interfere with MR-regulated renal sodium and potassium excretion.[8]

AZD9977 is a novel MR modulator that in preclinical studies exerted similar kidney protective effects as eplerenone, but in contrast to classical MRAs was devoid of acute effects on urinary $Na^+/K^+$ ratio, predicting a reduced hyperkalemia risk.[9] Furthermore, AZD9977 attenuated the natriuretic effect of eplerenone, suggesting that AZD9977 binds to kidney epithelial MR, but displays a

[1]Bioscience CKD, Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca, Sweden
[2]Drug Metabolism and Pharmacokinetics, Cardiovascular, Renal and Metabolism, IMED Biotech Unit, AstraZeneca, Sweden

**Corresponding author:**
Krister Bamberg, AstraZeneca, Pepparedsleden 1, 43183 Mölndal, Sweden.
Email: Krister.bamberg@astrazeneca.com

differentiated mode of action compared with classical MR antagonists.[9]

AZD9977 was recently evaluated in humans in whom it was well tolerated and displayed pharmacokinetics (PK) compatible with further development.[10] Effects on urine electrolytes were studied in healthy volunteers against the synthetic mineralocorticoid fludrocortisone. AZD9977 as well as eplerenone attenuated fludrocortisone-stimulated $Na^+$ retention, suggesting that both compounds yielded similar MR occupancy. Hence the study did not prove the pharmacological differentiation of AZD9977 vs eplerenone observed previously in rats when tested against the endogenous mineralocorticoid aldosterone.[9]

The aim of the current study is to test whether the observed discrepancy between effects of AZD9977 on urinary electrolyte excretion in humans and lack thereof in preclinical models is due to the mineralocorticoid used. To assess this, antimineralocorticoid effects of AZD9977 and eplerenone were compared in rats using either aldosterone or fludrocortisone as mineralocorticoids.

## Materials and methods

Experimental procedures to assess electrolytes effects in rats were approved by the local ethics review committee on animal experiments in the Gothenburg region (EA 141-2010). Fludrocortisone was purchased from Sigma-Aldrich, Germany, and aldosterone was purchased from Molekula Ltd, UK. Urinary $Na^+$ and $K^+$ concentrations were determined on a Radiometer ABL 700 blood gas analyzer (Radiometer, Copenhagen, Denmark).

### *Electrolyte effects in rats*

*Fludrocortisone experiment.* Vehicle or 100 μg/kg fludrocortisone was administered orally four times per rat at two-hour intervals, which yielded plasma exposures of fludrocortisone equivalent to what was achieved in the study in humans described in Erlandsson et al.[10] A rat PK study was carried out to set fludrocortisone doses: A total of 100 μg/kg fludrocortisone resulted in an average exposure of ~0.006 μM as in the clinical study.

Rats were placed in metabolic cages and 3, 10, 30 or 100 mg/kg AZD9977 or 1, 3, 10 or 30 mg/kg eplerenone was dosed two hours after the initial fludrocortisone administration by oral gavage (10 ml/kg). Urine was collected for six hours after AZD9977/eplerenone dosing and analyzed for $Na^+$ and $K^+$ content.

*Aldosterone experiment.* Rats were dosed via oral gavage with 2, 16, 54, 120 mg/kg AZD9977 or 1, 3, 10, 30, 100 mg/kg eplerenone (10 ml/kg) in a vehicle containing 2.7% NaCl. A total of 3 μg/kg aldosterone was administered subcutaneously within 15 minutes after dosing of either AZD9977 or eplerenone. Satellite animals were used to assess PK of aldosterone. The half-life of aldosterone was approximately 30 minutes and the average exposure during the first hour was ~0.003 μM. Aldosterone and fludrocortisone have similar in vitro potency as well as plasma protein binding and therefore exposures are directly comparable. Rats were placed in metabolic cages, and urine was collected for five hours after aldosterone administration and analyzed for $Na^+$ and $K^+$ content.

Satellite PK animals were used to assess drug exposure levels during the study duration with a total of 13 and 20 animals for AZD9977 and eplerenone, respectively. Maximum concentration ($C_{max}$) was between 10 minutes and one hour both for AZD9977 and eplerenone. Taking PK properties and in vitro potencies on rat MR into account, a three-times higher dose of AZD9977 is required to achieve the same receptor occupancy as previously described.[9]

### *Comparison of clinical exposure response data with preclinical data*

Clinical PK data previously presented by Erlandsson and colleagues[10] were used to obtain the average exposure during the eight-hour study period after dosing to assess clinical vs preclinical urinary electrolyte effects at similar exposure levels. The clinical average plasma concentration was converted to unbound exposure levels using human plasma protein binding (18% unbound for AZD9977, 51% for eplerenone) and then divided by the in vitro potency to account for any in vitro potency difference between AZD9977 and eplerenone.[9,10]

### *Data and statistical analysis*

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology.[11] Data are presented as group average ± SEM. Statistical analysis was by analysis of variance followed by Dunnett testing for multiple comparisons using Analyse-it for Microsoft Excel 4.51 (Analyse-it Software, Ltd).

## Results

### *Fludrocortisone as mineralocorticoid*

Fludrocortisone administration resulted in a reduced urinary $Na^+/K^+$ ratio (Figure 1), mainly as a result of reduced $Na^+$ excretion (S1 Figure). Using fludrocortisone as the mineralocorticoid, AZD9977 as well as eplerenone caused dose-dependent increases in urinary $Na^+/K^+$ ratio (Figure 1) as a result of increased $Na^+$ excretion (S1 Figure). At the highest dose, eplerenone but not AZD9977 treatment increased the urinary $Na^+/K^+$ ratio above baseline, but exposures at these doses were well above the

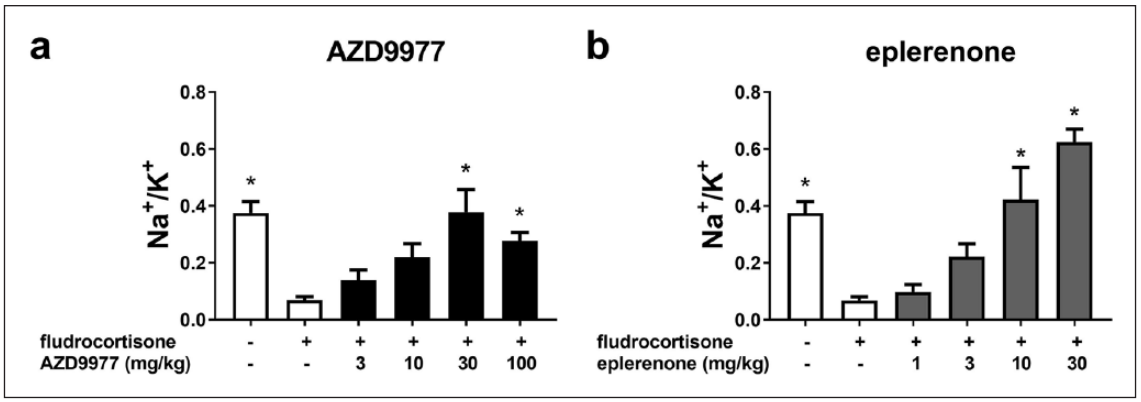


**Figure 1.** Repeated doses of fludrocortisone reduce the $Na^+/K^+$ ratio in urine collected two to eight hours after the initial dose. Single doses of (a) AZD9977 or (b) eplerenone reverse the effect. Doses in mg/kg are indicated below each bar. Mean ± SEM, $n = 7–31$ per group, $^*p < 0.05$ vs fludrocortisone alone. Data are assembled from four experiments.

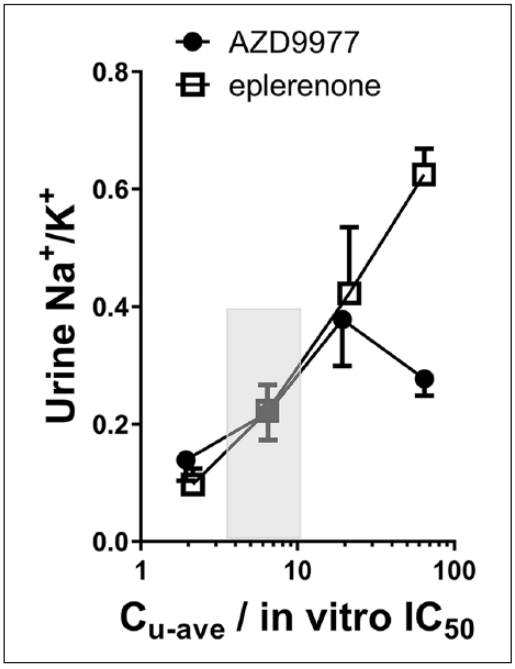


**Figure 2.** AZD9977 and eplerenone reversed the fludrocortisone-mediated effect on urinary $Na^+/K^+$ ratio equally efficaciously in human and rat at corresponding drug exposure levels. The responses illustrated in Figure 1 are plotted against achieved exposures of unbound ligand in relation to rat mineralocorticoid receptor (MR) in vitro $IC_{50}$ values ($C_{u-ave}$/in vitro $IC_{50}$). The grayed area illustrates the achieved exposure range of unbound ligand in relation to human MR in vitro $IC_{50}$ achieved in the human study.[10]

clinical exposure levels studied by Erlandsson et al.[10] (Figures 1 and 2). $K^+$ excretion was slightly elevated after fludrocortisone administration, but not altered by either AZD9977 or eplerenone dosing (S1 Figure). This result is in sharp contrast to the observations in rats with elevated endogenous aldosterone, in which eplerenone, but not AZD9977, affected urinary $Na^+/K^+$ ratio.[9]

### *Aldosterone as mineralocorticoid*

To exclude that the observed effects on urinary electrolytes were due to different protocols (exogenous fludrocortisone vs endogenous aldosterone), we assessed the effects of the compounds in an established model using exogenous aldosterone administration in combination with an NaCl load to increase the therapeutic window.[12] When eplerenone or AZD9977 were administered together with a subcutaneous aldosterone dose and NaCl load, eplerenone caused a dose-dependent increase of the urinary $Na^+/K^+$ ratio while AZD9977 did not, despite drug exposures yielding full receptor coverage (Figure 3). The increased urinary $Na^+/K^+$ ratio after eplerenone treatment was a result of increased $Na^+$ excretion, since urinary $K^+$ excretion was not measurably altered under these conditions (S2 Figure). Similar results were obtained when aldosterone was administered repeatedly and in absence of the NaCl load (S3 Figure). These results are in agreement with the observations in rats with elevated endogenous aldosterone, in which only eplerenone, but not AZD9977, affected the urinary $Na^+/K^+$ ratio.[9]

## Discussion

Despite clinical trial evidence demonstrating that MRAs reduce mortality and hospitalization in HF patients and a class I recommendation to use MRAs for treatment of HF with reduced ejection fraction, MRAs are among the most underutilized therapy for HF treatment.[13] This is largely because of the mechanism-based risk for hyperkalemia, which is mainly mediated through the effects of MRAs on urinary electrolyte excretion.

Acute effect on urinary $Na^+/K^+$ ratio is an established translational biomarker for MR antagonism[13] and is frequently used to characterize MRAs.[14–18] Preclinically, acute effects of MRAs on urinary $Na^+/K^+$ are typically assessed in presence of elevated levels of the physiological mineralocorticoid aldosterone,[12,17,18] while the synthetic,

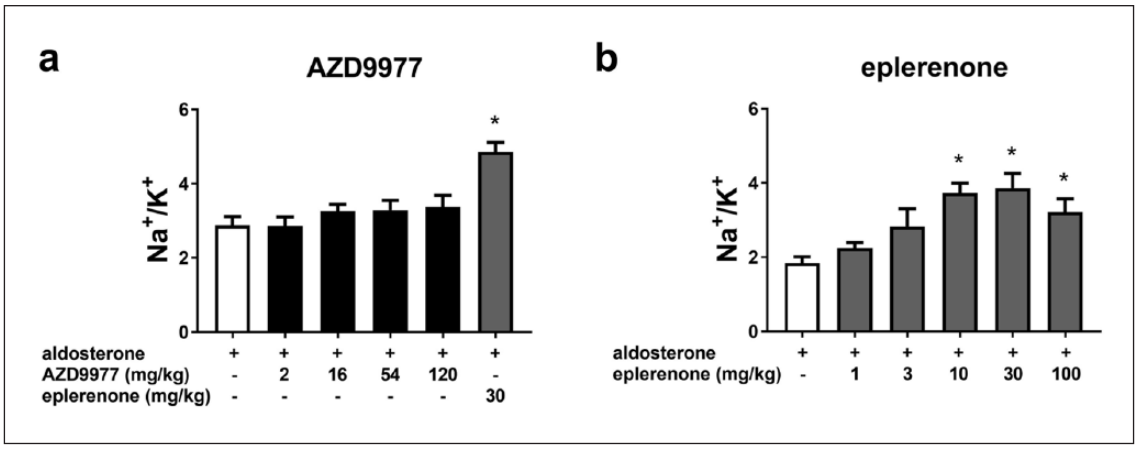


**Figure 3.** (a) AZD9977 does not affect the urinary $Na^+/K^+$ ratio after administration of an NaCl bolus and a single dose of aldosterone whereas ((b) and gray bar in (a)) eplerenone dose-dependently elevates the urinary $Na^+/K^+$ ratio. Doses in mg/kg are indicated below each bar. Mean ± SEM, $n = 7–8$ per group, $^*p < 0.05$ vs aldosterone alone. Data are assembled from two experiments.

orally available MR agonist fludrocortisone is used to activate MR in corresponding clinical trials.[19] Studies comparing fludrocortisone with aldosterone and the pharmacological effects of MRAs in the presence of either fludrocortisone or aldosterone suggested that fludrocortisone apart from its oral bioavailability and prolonged half-life displays similar properties as aldosterone.[20–23]

The novel MR modulator AZD9977 is differentiated from classical MRAs since it does not alter acute urinary electrolyte excretion over a wide dose range in the presence of constantly elevated endogenous aldosterone levels in preclinical testing.[9]

Unexpectedly, when effects of AZD9977 on acute urinary electrolyte excretion were evaluated in a clinical trial in the presence of fludrocortisone, administration of AZD9977 increased the urinary $Na^+/K^+$ ratio to a similar extent as eplerenone.[10] While the study did not prove that AZD9977 lacks effect on acute urinary electrolyte handling in humans, it did support that AZD9977 interacts with MR at expected therapeutic exposure levels.

To evaluate whether the unexpected effect of AZD9977 on urinary electrolytes seen in the clinical study is related to the use of fludrocortisone, we conducted preclinical studies with fludrocortisone in rats using a protocol that matched the clinical trial protocol. As shown here, AZD9977 dose-dependently increased urinary $Na^+/K^+$ ratio also in rats when tested against fludrocortisone.

Modeling of preclinical and clinical data suggest that the observed acute effects of eplerenone and AZD9977 on urinary electrolytes in the presence of fludrocortisone quantitatively translate from rats to humans. In the preclinical study using fludrocortisone, at drug exposure levels corresponding to those assessed clinically,[10] AZD9977 and eplerenone dosing resulted in an approximate two- to three-fold increased urinary $Na^+/K^+$ ratio compared with rats receiving fludrocortisone only (Figures 1 and 2), which was a similar increase as observed in humans. Thus, eplerenone and AZD9977 are equally efficacious in humans and rats with regard to inhibiting fludrocortisone-induced $Na^+$ retention in the exposure range achieved in the clinical study (Figure 2). At exposures in rats exceeding the achieved clinical exposures, eplerenone, but not AZD9977, causes a further increase in urinary $Na^+/K^+$ ratio. This suggests that AZD9977 may act as a partial antagonist whereas eplerenone acts as a full antagonist upon fludrocortisone administration.

A different experimental protocol was used to study the effects of AZD9977 and eplerenone in the presence of fludrocortisone using administration of exogenous fludrocortisone as compared with previously described experiments using endogenous aldosterone elevations.[9] We therefore extended the previous studies with aldosterone in rats and show here that AZD9977 lacks effects on acute electrolyte excretion also when tested against exogenously administered aldosterone. The data presented here suggest that the key difference regarding effects of AZD9977 on urinary electrolytes between the preclinical studies with aldosterone and clinical studies using fludrocortisone are related to the use of different MR agonists rather than species differences or differences in experimental setup. Despite the overall similar physiological effects of aldosterone and fludrocortisone, the use of AZD9977 and eplerenone highlight that fludrocortisone appears to affect urinary $Na^+$ handling differently from aldosterone. To our knowledge, there are no publicly available data to date describing disparate molecular genomic or nongenomic effects of fludrocortisone compared with aldosterone. At the exposures achieved in this study, fludrocortisone can also activate glucocorticoid receptors, which may contribute to the observed differences in urinary electrolyte handling. Further studies are warranted to explore the molecular mechanism behind the differentiated effects of aldosterone and fludrocortisone.

The minimal urinary electrolyte effects of AZD9977 observed in rats in the presence of the physiological MR ligand aldosterone may therefore translate to a negligible urinary electrolyte effect for AZD9977 at therapeutic exposure in patients as well and support that AZD9977 may be superior to classical MRAs with regards to potassium retention. Further clinical trials will clarify whether AZD9977 treatment in patients has a reduced effect on plasma potassium compared with MRAs.

## Conclusions

The presented data using the MR modulator AZD9977 suggest different mineralocorticoid effects of the synthetic mineralocorticoid fludrocortisone compared with the physiological mineralocorticoid aldosterone. The data suggest that fludrocortisone-based pharmacodynamic clinical studies may be appropriate for testing traditional MRAs as previously argued,[13] but studies assessing MR modulators should be conducted in the presence of aldosterone. While fludrocortisone studies in healthy volunteers translate reasonably well to potassium retention in patients on MRAs,[18] such translation for MR modulators may not be as straightforward.

### Acknowledgements

We want to thank Malin Antonsson for expert animal handling during the study and Caroline Wingolf for formulating all dose solutions.

### Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: The authors are employees of AstraZeneca and take complete responsibility for the integrity of the data and accuracy of the data analysis.

### Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was funded by AstraZeneca. Krister Bamberg, Lena William-Olsson, Ulrika Johansson, Rasmus Jansson-Löfmark and Judith Hartleib-Geschwindner are employed by AstraZeneca.

### Supplemental material

Supplemental material for this article is available online.

### ORCID iD

Krister Bamberg https://orcid.org/0000-0002-0538-6083

### References

1. Bauersachs J, Jaisser F and Toto R. Mineralocorticoid receptor activation and mineralocorticoid receptor antagonist treatment in cardiac and renal diseases. *Hypertension* 2015; 65: 257–263.
2. Bertocchio JP, Warnock DG and Jaisser F. Mineralocorticoid receptor activation and blockade: An emerging paradigm in chronic kidney disease. *Kidney Int* 2011; 79: 1051–1060.
3. Pitt B, Remme W, Zannad F, et al. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *NEngl J Med* 2003; 348: 1309–1321.
4. Zannad F, McMurray JJ, Krum H, et al. Eplerenone in patients with systolic heart failure and mild symptoms. *NEngl J Med* 2011; 364: 11–21.
5. Bakris GL, Agarwal R, Chan JC, et al. Effect of finerenone on albuminuria in patients with diabetic nephropathy: A randomized clinical trial. *JAMA* 2015; 314: 884–894.
6. Kolkhof P, Nowack C and Eitner F. Nonsteroidal antagonists of the mineralocorticoid receptor. *Curr Opin Nephrol Hypertens* 2015; 24: 417–424.
7. Gomez-Sanchez EP. Third-generation mineralocorticoid receptor antagonists: Why do we need a fourth? *J Cardiovasc Pharmacol* 2016; 67: 26–38.
8. Palmer BF. Managing hyperkalemia caused by inhibitors of the renin-angiotensin-aldosterone system. *NEngl J Med* 2004; 351: 585–592.
9. Bamberg K, Johansson U, Edman K, et al. Preclinical pharmacology of AZD9977: A novel mineralocorticoid receptor modulator separating organ protection from effects on electrolyte excretion. *PLoS One* 2018; 13: e0193380.
10. Erlandsson F, Albayaty M, Chialda L, et al. Clinical safety, tolerability, pharmacokinetics and effects on urinary electrolyte excretion of AZD9977, a novel, selective mineralocorticoid receptor modulator. *Br J Clin Pharmacol* 2018; 84: 1486–1493.
11. Curtis MJ and Abernethy DR. Advice on statistical analysis, and new journal guidance for experimental design and analysis. *Pharmacol Res Perspect* 2015; 3: e00095.
12. Brandish PE, Chen H, Szczerba P, et al. Development of a simplified assay for determination of the antimineralocorticoid activity of compounds dosed in rats. *J Pharmacol Toxicol Methods* 2008; 57: 155–160.
13. Dev S, Hoffman TK, Kavalieratos D, et al. Barriers to adoption of mineralocorticoid receptor antagonists in patients with heart failure: A mixed-methods study. *J Am Heart Assoc* 2016; 5: e002493.
14. Eudy RJ, Sahasrabudhe V, Sweeney K, et al. The use of plasma aldosterone and urinary sodium to potassium ratio as translatable quantitative biomarkers of mineralocorticoid receptor antagonism. *J Transl Med* 2011; 9: 180.
15. Nariai T, Fujita K, Mori M, et al. SM-368229, a novel selective and potent non-steroidal mineralocorticoid receptor antagonist with strong urinary $Na^+$ excretion activity. *J Pharmacol Sci* 2011; 115: 346–353.
16. Arai K, Tsuruoka H and Homma T. CS-3150, a novel nonsteroidal mineralocorticoid receptor antagonist, prevents hypertension and cardiorenal injury in Dahl salt-sensitive hypertensive rats. *EurJ Pharmacol* 2015; 769: 266–273.
17. Kagawa CM, Sturtevant FM and Van Arman CG. Pharmacology of a new steroid that blocks salt activity of aldosterone and desoxycorticosterone. *J Pharmacol Exp Ther* 1959; 126: 123–130.
18. Bärfacker L, Kuhl A, Hillisch A, et al. Discovery of BAY 94-8862: A nonsteroidal antagonist of the mineralocorticoid

receptor for the treatment of cardiorenal diseases. *Chem MedChem* 2012; 7: 1385–1403.
19. Ramsay L, Harrison I, Shelton J, et al. Relative potency of prorenoate and spironolactone in normal man. *Clin Pharmacol Ther* 1975; 18: 391–400.
20. Ross EJ. Human assay of electrolyte-active steroids and their antagonists. *Clin Sci* 1962; 23: 197–202.
21. Genard P and Palem-Vliers M. Effect of 6-dehydro-DOCA and 6-dehydro-9 alpha-fluorocortisol acetate on the excretion of sodium and potassium in the rat. *J Steroid Biochem* 1985; 23: 673–675.
22. Thorn GW, Sheppard RH, Morse WI, et al. Comparative action of aldosterone and 9-alpha-fluorohydrocortisone in man. *Ann N Y Acad Sci* 1955; 61: 609–619.
23. Ramsay LE, Hessian P and Tidd MJ. Bioassay of aldosterone antagonists in normal human subjects: A relationship between the level of plasma uric acid before treatment and apparent drug responses. *Br J Clin Pharmacol* 1975; 2: 271–276.